

tho I hGH Signal Sequence

hGH Intron

TCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACATGTGTCCTGAGGGTAGAGGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGGGCTT-T

Met Ala Thr

CONTROL CONTRO

CAR GRG GGC AGT GCC GAT AGT GTG TGT CCC CAR GGA ARA TAT ATC CAC CCT CAR ART ART TCG ATT TGC TGT ACC ARG TGC CAC ARA GGA
GIN GIU GIy Ser Ala Asp Ser Vai Cys Pro Gin Giy Lys Tyr lie His Pro Gin Asn Asn Ser lie Cys Cys Thr Lys Cys His Lys Giy

ACC TAC THE TAC AAT GAC TET CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TET GAG AGC GGC TCC TTC ACC GCT TCA GAA AAC CAC CTC

Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gin Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu

AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TCT TGC ACA GTG GAC CGG GAC ACC GTG TGT GGC TGC

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Giu Met Giy Gin Val Giu lie Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Giy Cys
AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC TGC TGC

Arg Lys Asn Gin Tyr Arg His Tyr Trp Ser Giu Asn Leu Phe Gin Cys Phe Asn Cys Ser Leu Cys Leu Asn Giy Thr Val His Leu Ser Cys Linker

CAG GAG AAA CAG ACC CTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT GCC GGT GCC CCA GGT
GIN Glu Lys Gin Asn Thr Vai Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Vai Ser Cys Ala Gly Ala Ala Pro Gly
+7 Cys of hCG alpha

TIGC CCA GAA TIGC ACG CTA CAG GAA AAC CCA TIC TIC TIC CAG CCG GGT GCC CCA ATA CIT CAG TIGC ATG GGC TIGC TIC TICT AGA GCA TAT

Cys Pro Glu Cys Thr Leu Gin Glu Asn Pro Phe Phe Ser Gin Pro Gly Ala Pro Ille Leu Gin Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr

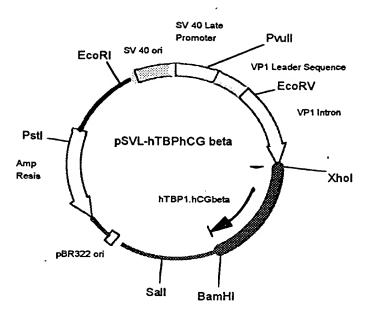
CCC ACT CCA CTA AGG TCC AAG AAG ACG ATG TTG GTC CAA AAG AAC GTC ACC TCA GAG TCC ACT TGC TGT GTA GCT AAA TCA TAT AAC AGG GTC
Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu Vai Gin Lys Asn Vai Thr Ser Giu Ser Thr Cys Cys Vai Aia Lys Ser Tyr Asn Arg Vai

ACA GTA ATG GGG GGT TTC AAA GTG GAG AAC CAC ACG GCG TGC CAC TGC AGT ATT TGT TAT TAT CAC AAA TCT TAA G

Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser Thr Cys Tyr His Lys Ser ...

Bam HI

Figure 1 (a)



hGH Signal Sequence

hGH Intron

Xhol

CTCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGAGGCCACCTGTAGATGGGACGGGGGGCACTAACCCTCAGGTTTGGG

Met Ala Thr

Ser Arg Thr Ser Leu Leu Ala Phe Gly Leu Leu Cys Leu +20 Asp of Proceesed TBP1

Pro Trp Leu Gin Giu Giy Ser Ala Asp Ser Val Cys Pro Gin Giy Lys Tyr He His Pro Gin Asn Asn Ser He Cys Cys Thr

AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC
Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gin Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr

GCT TCA GAA AAC CAC CTC AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TGC ACA GTG GAC
Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu IIe Ser Ser Cys Thr Val Asp

CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC GTC TGC CTC
Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gin Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gin Cys Phe Asn Cys Ser Leu Cys Leu

AAT GGG ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT ITC TTT CTA AGA GAA AAC GAG TGT GTC

ASn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val
Linker +7 Pro of hCG beta

TOO TOT GOT GOT GOT GOT COA CGG TGC CGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG GGC TGC CCC GTG TGC ATC ACC GTC
Ser Cys Ala Gly Ala Gly Pro Arg Cys Arg Pro lie Asn Ala Thr Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys lie Thr Val

AAC ACC ACC ATC TGT GCC GGC TAC TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT CAG GTG GTG TGC AAC TAC
ASn Thr Thr lie Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gin Gly Val Leu Pro Ala Leu Pro Gin Val Val Cys Asn Tyr

CGC GAT GTG CGC TTC GAG TCC ATC CGG CTC CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT CTC AGC TGT CAA
Arg Asp Vai Arg Phe Giu Ser lie Arg Leu Pro Giy Cys Pro Arg Gly Vai Asn Pro Vai Vai Ser Tyr Ala Vai Ala Leu Ser Cys Gin

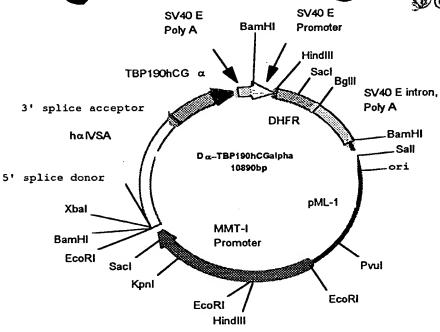
TOT GOA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC TCC TCT Cys Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Phe Gln Asp Ser Ser

TCC TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAA

Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gin · · ·

Bam HI

Figure 1 (b)



Xhol hGH Signal Sequence hGH Intron

TCGAG ATG GCT ACA G GTAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAAGGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGGGCTTCT

▶ Me t Ala Thr

CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG ATT TGC TGT ACC AAG TGC CAC AAA GGA

GLU GLU GLU Ser Ala Asp Ser Val Cys Pro Glu Gly Lys Tyr Ile His Pro Glu Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys Gly

ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC GCT TCA GAA AAC CAC CTC

Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu

AGA CAC TGC CTC AGC TGC TCC AAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TGT TGC ACA GTG GAC CGG GAC ACC GTG TGT GGC TGC
AGA CAC TGC CTC AGC TGC TGC CAAA TGC CGA AAG GAA ATG GGT CAG GTG GAG ATC TCT TGT TGC ACA GTG GAC CGG GAC ACC GTG TGT GGC TGC
ATG His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys

AGG AAG CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT GGG ACC GTG CAC CTC TGC TGC

AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC AAT GGG ACC GTG CAC CTC TGC

ATG Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys

CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC TCC TGT AGT AAC TGT AAG AAA AGC CTG

Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Ser Leu

Linker +7 Cys of hCG alpha

GAA TGC ACG CTA CAG GAA AAC CCA TTC TTC TCC CAG CCG GGT GCC CCA ATA CTT CAG TGC ATG GGC TGC TGC TTC TCT AGA GCA TAT CCC ACT

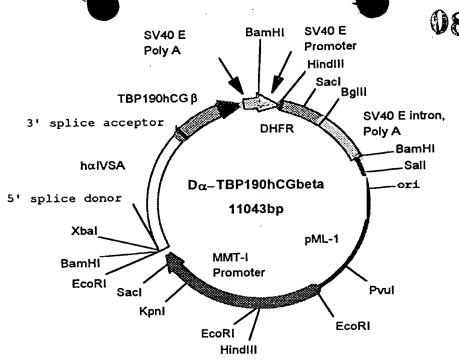
Glu Cys Thr Leu Gln Glu Asn Pro Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr

CCA CTA AGG TCC AAG AAG ACG ATG TTG GTC CAA AAG AAC GTC ACC TCA GAG TCC ACT TGC TGT GTA GCT AAA TCA TAT AAC AGG GTC ACA GTA
Pro Leu Arg Ser Lys Lys Thr Met Leu Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser Tyr Asn Arg Val Thr Val

ATG GGG GGT TTC AAA GTG GAG AAC CAC ACG GCG TGC CAC TGC AGT ACT TGT TAT TAT CAC AAA TCT TAA GGATCCCTCGAG

Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser · · · BamHl Xhol

Figure 2(a)
TBP(20-190)-hCGα FUSION CONSTRUCT



Xhol hGH Signal Sequence hGH Intron

CTCGAG ATG GCT ACA G GTAAGCGCCCCTAAAATCCCTTTGGGCACAATGTGTCCTGAGGGGAGGAGGCAGCGACCTGTAGATGGGACGGGGGCACTAACCCTCAGGTTTGGG

Met Ala Thr

CTCTTGCTCTCCGGCTCCCTCTGTTGCCCTCTGGTTTCTCCCCAGGC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC CTG

Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys Leu

+20 Asp of Proceesed TBP1 CCC TGG CTT CAA GAG GGC AGT GCC GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TGG ATT TGC TGT ACC Pro Trp Leu Gln Glu Gly Ser Ala Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr AAG TGC CAC AAA GGA ACC TAC TTG TAC AAT GAC TGT CCA GGC CCG GGG CAG GAT ACG GAC TGC AGG GAG TGT GAG AGC GGC TCC TTC ACC Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr GCT TOA GAA ARD CAC CTO RGA CAC TGC CTC AGO TGC TCC ARA TGC CGA ARG GAA ATG GGT CAG GTG GAG ATC TCT TGT TGC ACA GTG GAC Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp CGG GAC ACC GTG TGT GGC TGC AGG AAG AAC CAG TAC CGG CAT TAT TGG AGT GAA AAC CTT TTC CAG TGC TTC AAT TGC AGC CTC TGC CTC Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu AAT GGG ACC GTG CAC CTC TCC TGC CAG GAG AAA CAG AAC ACC GTG TGC ACC TGC CAT GCA GGT TTC TTT CTA AGA GAA AAC GAG TGT GTC Asn Gly Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val TCC TGT AGT AAC TGT AAG AAA AGC CTG GAG TGC ACG AAG TTG TGC CTA CCC CAG ATT GAG AAT GTT AAG GGC ACT GAG GAC TCA GGC ACC Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser Gly Thr +7 Pro of beta Linker ACA GCT GGT GCT CCA CGG TGC CCC ATC AAT GCC ACC CTG GCT GTG GAG AAG GAG GGC TGC CCC GTG TGC ATC ACC GTC AAC The Ala Gly Ala Gly Pro Arg Cys Arg Pro Ile Asn Ala The Leu Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile The Val Asn ACC ACC ATC TGT GCC GGC TAC TGC CCC ACC ATG ACC CGC GTG CTG CAG GGG GTC CTG CCG GCC CTG CCT CAG GTG GTG TGC AAC TAC CGC Thr Thr Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg GAT GTG CGC TTC GAG TCC ATC CGG CTC CCT GGC TGC CCG CGC GGC GTG AAC CCC GTG GTC TCC TAC GCC GTG GCT CTC AGC TGT CAA TGT Asp Val Arg Phe Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys

Figure 2 (b)
TBP(20-190)-hCGβ FUSION CONSTRUCT

GCA CTC TGC CGC CGC AGC ACC ACT GAC TGC GGG GGT CCC AAG GAC CAC CCC TTG ACC TGT GAT GAC CCC CGC TTC CAG GAC TCC TCT TCC

Ala Leu Cys Arg Arg Ser Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp Pro Arg Fhe Glm Asp Ser Ser Ser

TCA AAG GCC CCT CCC CCC AGC CTT CCA AGC CCA TCC CGA CTC CCG GGG CCC TCG GAC ACC CCG ATC CTC CCA CAA TAA GGATCCCTCGAG

Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln · · ·

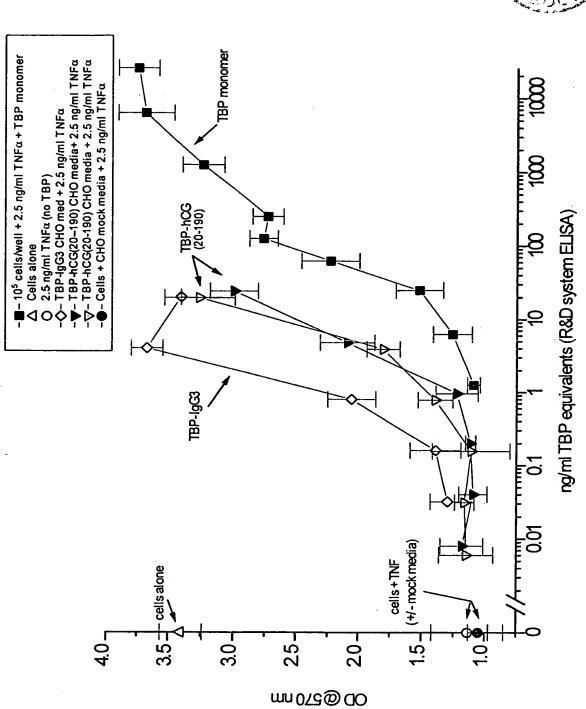


Figure 5. COS cell expressed TBP-hCG(20-190) inhibits TNF definated cytotoxicity on BT-20 cells

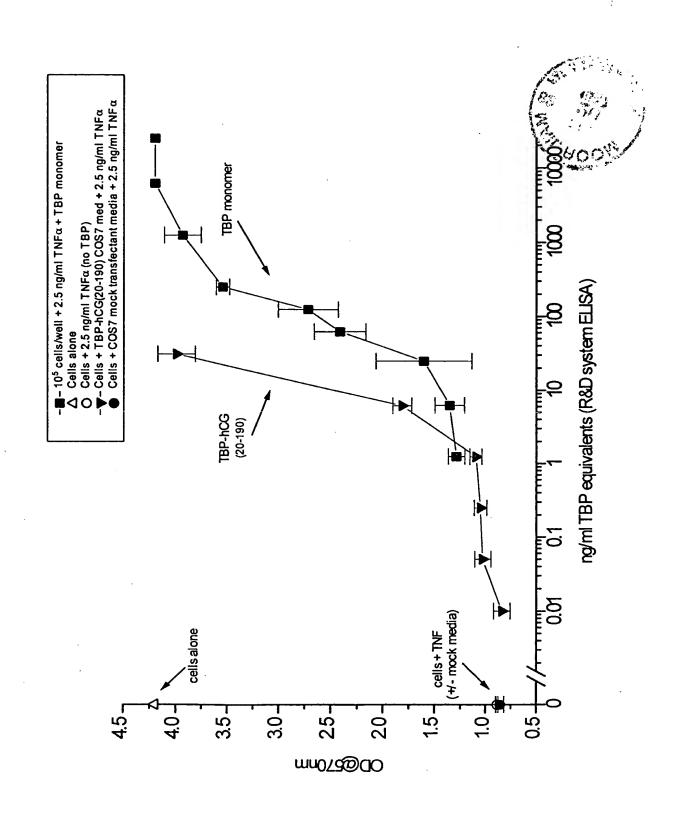
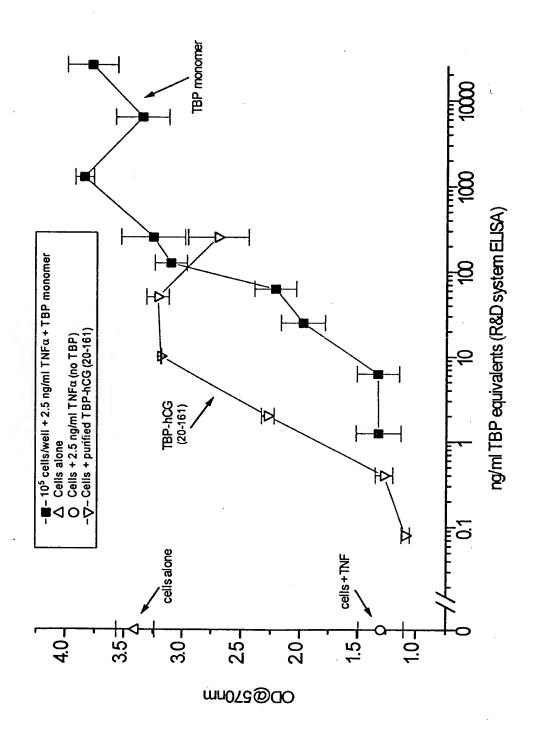


Figure 6. Affinity purified CHO cell expressed TBP-hCG(20-1615) and inhibits TNFα-induced cytotoxicity on BT-20 cells



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